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ORIGINAL ARTICLE

Alterations of human electroencephalographic activity caused by multiple extremely low frequency magnetic field exposures

Dean Cvetkovic · Irena Cosic

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Abstract In the past, many studies have claimed that extremely low frequency (ELF) magnetic field (MF) exposures could alter the human electroencephalographic (EEG) activity. This study aims at extending our ELF pilot study to investigate whether MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within the corresponding EEG bands. 33 human subjects were tested under a double-blind and counter-balanced conditions. The multiple repeated three-way analysis of variance (ANOVA) mixed design (within and between-subject) analysis was employed followed by post-hoc *t*-tests and Bonferroni alpha-correction. The results from this study have shown that narrow alpha1 (7.5–9.5 Hz) and alpha2 (9–11 Hz) bands, associated with 8.33 and 10 Hz MF exposures, were significantly ($p < 0.0005$) lower than control over the temporal and parietal regions within the 10–16 min of first MF exposure session and the MF exposures were significantly higher than control of the second session MF exposure (60–65 min from the commencement of testing). Also, it was found that the beta1 (12–14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at frontal region. The final outcome of our result has shown that it is possible to alter the human EEG activity of alpha and beta bands when exposed to MF at frequencies corresponding to those same bands, depending on the order and period of MF conditions. This type of EEG synchronisation of driving alpha and beta EEG by alpha and beta sinusoidal MF stimulation, demonstrated in this study, could possibly be applied as therapeutic treatment(s) of particular neurophysiological abnormalities such as sleep and psychiatric disorders.

Keywords EEG · ELF · Magnetic field · Stimulation · ANOVA

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1 Introduction

In most bioelectromagnetics studies which examined the effects of extremely low frequency (ELF) magnetic fields (MF) upon the human electroencephalographic (EEG) activity, there have been inconsistencies in findings between experiments [4], due to differences in the experimental protocols, electromagnetic field characteristics, pulse shape, spatial characteristics, frequency, period of exposure and organism itself. The ELF research is still surrounded by a fair share of controversy within the scientific and general communities, despite extensive research during the past several decades in this area.

Over the last two decades, several ELF studies have claimed that MF exposure characteristics could alter the human EEG activity, as follows: a sinusoidal 60-min intermittent MF exposure of 45 Hz and 1.26 mT [14]; a 2-s MF exposure of 60 Hz/20–100 μT [2]; 2-s epochs exposed to MF exposure of 10 Hz/40 μT and 1.5 Hz/20 μT [3]; a 2-s light and MF exposures to 1.5 Hz and 10 Hz/80 μT [15]; a 16 Hz/28.3 μT MF exposure [19]; an intermittent 16.7-Hz MF exposure [12]; a 2-s 60 Hz/100 μT MF exposure followed by 5-s control [16]; a 90-min MF exposure 50 Hz/80 μT [11]; and a 8.3 Hz–12.2 Hz/5 μT sinusoidal MF exposure frequencies corresponding to recorded frontal EEG signals [20]. Other studies have investigated the effects of ELF pulsed electromagnetic fields (PEMF) on EEG activity after the applied *Thomas pattern* signal

(0–500 Hz) pulses in 853-ms segments (18 pulses) at various periods of 110, 220, 330 ms at ±200 μT [4, 5, 21]; 'during' an ELF PEMF exposure [6]. The EEG responses to these MF exposures are described and discussed in Results' section of this article.

The authors' pilot studies examined the effects of sinusoidal 8.33 Hz/174 μT ELF MF [7, 8]. These results have led the authors to further investigate the EEG activity alterations due to MF exposures at frequencies associated with EEG bands. For example, authors' pilot study [8] has revealed a marginal significant decrease in MF exposure compared to control, found in the alpha1 EEG band (7.5–9.5 Hz) at the vertex head position, where MF exposure was applied at the alpha1 frequency of 8.33 Hz/174 μT.

This study aims at extending our ELF pilot study to investigate whether MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within its corresponding EEG bands.

2 Materials and methods

2.1 Subjects

The experiments were conducted on 33 healthy subjects (24 male and 9 female) with mean age of 30 years, SD 11 years, range 20–59 years. The RMIT University's ethics committee approved the study, and all the subjects gave written informed consent prior to the experiment.

2.2 Magnetic field exposure system

A pair of standard circular Helmholtz coils have been designed by the authors, having a driving current of 140 mA, total coil impedance 71 Ω, average radius of 65 cm, copper wire of 0.8 mm in diameter and 250 turns each (Fig. 1) [9]. A signal generator effective in producing high quality sine waveforms of high stability/accuracy ELF signals was designed and developed using EXAR XR-2206 monolithic IC together with an audio amplifier with the gain of 10 to deliver sufficient current level to the coils. The magnetic flux density was verified by direct measurement using 'Wandel and Goltermann' EFA-200 EMF Analyser. The linearly polarised field was perpendicular to the Earth's North–South MF at magnetic flux density of 20 μT (rms). The Helmholtz coils were designed and constructed to ensure the matching of source impedance with the coil reactance, exact series inductance and mutual inductance between the coils of the pair. Uniformity levels from the inner to the outer region were 0.01, 0.1 and 1% with respect to the centre value. According to magnetic flux density measurements acquired, the uniformity of the inner level, 0.01%, was 15 cm (x- and y-axis) and the outer level 1% was 40 cm (x-axis) and 50 cm (y-axis) [9]. The measured ambient or geomagnetic field inside the RF-shielded room was approximately 200 nT at the ELF range 6–11 Hz.

2.3 Electroencephalogram

The EEG data acquisition equipment used throughout testing was the Mindset MS-1000 (Nolan Computer Systems Inc., USA) recording system. Neuroscan 19 channel cap (Compumedics Neuroscan Limited, USA) electrodes were used with referential montage of 16 channels. The left brain hemisphere electrodes: Fp1, F7, F3, T7, C3, P7, P3 and O1 were all referenced to M1 (left mastoid), while the right brain hemisphere electrodes: Fp2, F8, F4, T8, C4, P8, P4 and O2 were referenced to right mastoid M2.

2.4 Experimental procedure

During the EEG recording sessions, subjects were asked to lie down between the coils in sagittal plane direction perpendicular to the coil axis and in the supine position. The entire experiment was performed in a darkened, sound

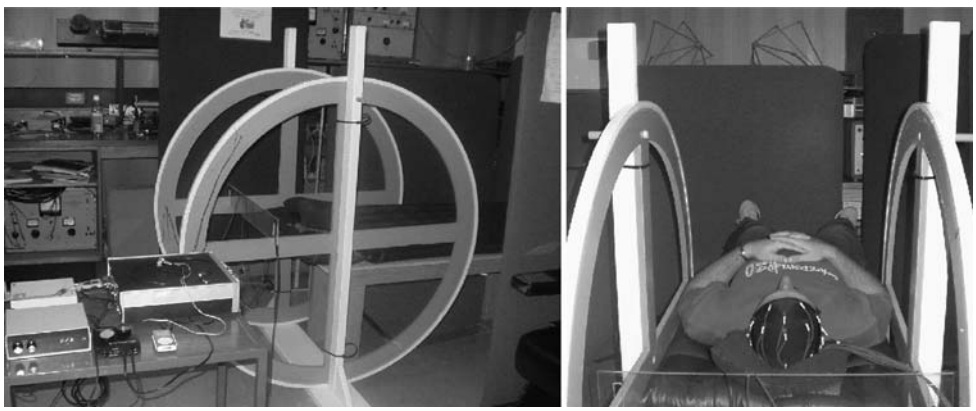

Fig. 1 The design of magnetic field exposure system consisting of Helmholtz coils and a signal generator

proof and RF-shielded room to prevent erroneous recordings due to the standing waves and power line interference.

The baseline EEG was recorded prior to any stimulation for one minute. Each stimulation (50, 16.66, 13, 10, 8.33 and 4 Hz) lasted for 2 min followed by 1 min EEG recording, as shown in Fig. 2. The total duration of an experiment was 19 min. The same procedure was repeated for the 'no MF' or control sessions. The order of control and exposure sessions was determined randomly according to the subject's ID number. Subjects with odd ID numbers were first tested with control condition (no MF exposure) followed by MF exposure after a 30-min break. Double-blind and counter-balanced condition was exercised. This condition was highly considered in the analysis as a factor that might reveal that if the first session was an MF exposure, the EEG activity results during the second MF control session could still be influenced or dependent on the results of the first MF exposure session.

The MF study protocol consisted of:

- first EEG baseline recording, followed by
- first MF exposure/control at 50 Hz; continued by second EEG recording (after 50 Hz);
- second MF exposure/control at 16.66 Hz; third EEG recording of after 16.66 Hz;
- third MF exposure/control at 13 Hz; fourth EEG recording (after 13 Hz);
- fourth MF exposure/control at 10 Hz; fifth EEG recording (after 10 Hz);
- fifth MF exposure/control at 8.33 Hz; sixth EEG recording (after 8.33 Hz);
- sixth MF exposure/control at 4 Hz; and seventh EEG recording (after 4 Hz).

The narrow EEG band intervals were as theta (3–5 Hz), alpha1 (7.5–9.5 Hz), alpha2 (9–11 Hz), beta1 (12–14 Hz), beta2 (15.5–17.5 Hz) and gamma (49–51 Hz). The theta and gamma band data was excluded from this particular analysis. We compared the EEG activity 'before' and 'after' stimulation for each frequency stimulation and band. The traditional EEG band definitions were not considered in this study. However, the EEG bands were custom defined around the stimulation frequency acting similar to a central frequency. For example, the stimulation of 8.33 Hz (close to 8.5 Hz) could investigate only within the theta band of ±1 Hz, which explains the 7.5–9.5 Hz range. The same procedure is repeated for other stimulation frequencies. It is important to point out that the EEG bands were defined based on the stimulation frequency parameters and not vice versa.

The computed extracted parameters were: the total spectral power of each stimulation EEG data (i.e. before, 50, 16.66, 13, 10, 8.33 and 4 Hz); spectral power in the stimulated band, before/after; central band frequency before/after; and relative difference 'ratio' between the individual band and total spectral power before/after. The relative spectral power estimate of each narrow frequency band corresponds to its own stimulus frequency, as described in Fig. 2. For example:

- gamma EEG band (49–51 Hz) relative power was calculated within the first EEG baseline recording and second EEG recording (after 50 Hz);
- beta2 EEG band (15.5–17.5 Hz) relative power within second EEG recording (after 50 Hz) and third EEG recording of after 16.66 Hz;
- beta1 EEG band (12–14 Hz) relative power within third EEG recording (after 16.66 Hz) and fourth EEG recording of after 13 Hz;

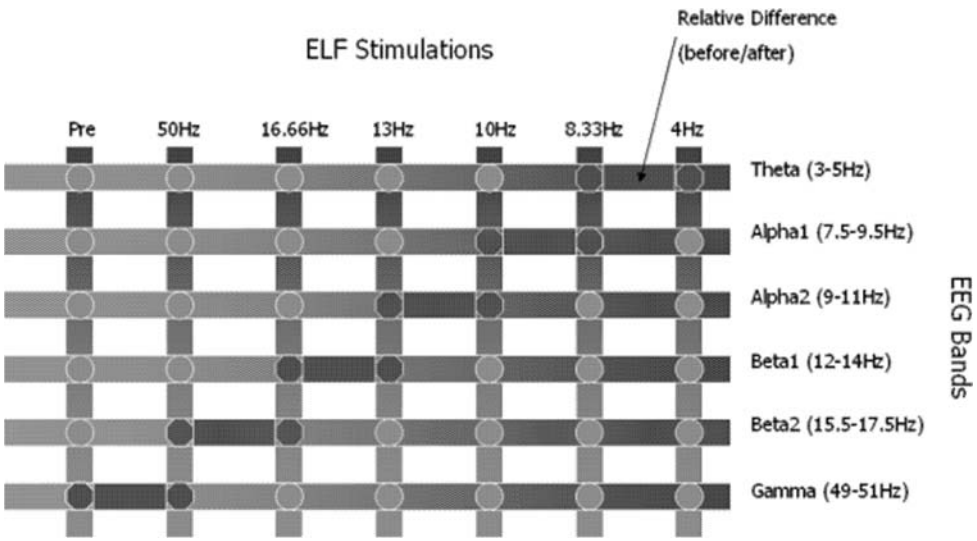

Fig. 2 The design of EEG measurement and MF condition protocol was instrumental in the design of the original data analysis method applied in this study. The analysis method consisted of computing the relative spectral power or the 'power ratio' between the individual band and total spectral band before and after MF condition. The relative spectral power estimate of each frequency band corresponds to its own stimulus frequency. The dark colour circles indicate the computed EEG data

- alpha2 EEG band (9–11 Hz) relative power within fourth EEG recording (after 13 Hz) and fifth EEG recording of after 10 Hz;
- alpha1 EEG band (7.5–9.5 Hz) relative power within fifth EEG recording (after 10 Hz) and sixth EEG recording of after 8.33 Hz;
- theta EEG band (3–5 Hz) relative power within sixth EEG recording (after 8.33 Hz) and seventh EEG recording of after 4 Hz.

The advantage of applying this particular method by only analysing the stimulus frequency EEG band was to simplify and reduce the number of multiple statistical tests in the analysis. Throughout the statistical analysis of this study, the gamma EEG band at MF stimulus of 50 Hz data was excluded from the analysis due to an interest in EEG frequency responses, less than 50 Hz.

2.5 Signal processing

All the collected EEG data was processed using MATLAB (Mathworks, USA) employing the Short Time Fourier Transform (STFT) function to compute the spectral analysis of all the 16 channels for all the subjects and extract parameters to be used in the statistical analysis. The EEG power was calculated by integrating the area under the curve at specific frequency band intervals. The EEG power spectra were computed with the resolution of 0.5 Hz with 60 epochs (1 s for each epoch) for 60-s recording with sampling frequency of 256 Hz.

3 Results

All-subject processed data were statistically analysed using statistical SPSS software tool (SPSS, Statistical Packages for Social Sciences, version 14, SPSS, Inc., Chicago, IL, USA). Initially, multiple repeated three-way analysis of variance (ANOVA) mixed design (within and between-subject) tests, with a significant level set at 0.05, were conducted. The factorial designs were used to evaluate the possible existence of the main effect such as: within-subject 'condition' (exposure/control) and 'order' (before/after); and between-subject 'session' (first/second); and its combined effects or interaction between factors, such as: 'condition × order', 'condition × session' and 'order × sessions'. Following the multiple ANOVA tests, the post-hoc analysis was conducted using multiple paired samples two-tailed *t*-tests. The first test conducted was for the first session of MF exposure consisting of 16 subjects (df = 15). The second test was the second session MF control (df = 15), the third test was the first session MF control (df = 16) and the fourth test was the second session MF exposure (df = 16). The following two sub-sections will describe the results, initially describing the exposure followed by control 'order' and control followed by the exposure 'order'.

3.1 MF exposure followed by MF control results

In alpha1 band, under 8.33 Hz, second MF control session (no field) *t*-test results revealed a significant increase from before to after at T7 ($t_{15} = -2.397$, $p < 0.030$) (Fig. 3). ANOVA test revealed a significant difference for the interaction between exposure/control and sessions factors (T7) $F_{1,31} = 5.992$, $p < 0.020$ (Table 1). In alpha2 band 10 Hz MF second control session (P3), there was a significant decrease from before [mean (M) = 0.1789, standard error (SE) = 0.0201] to after ($M = 0.1573$, SE = 0.0140), $t_{15} = 3.081$, $p < 0.008$) (Fig. 4). At P4 alpha2 band, the significant decrease from before ($M = 0.1861$, SE = 0.0223) to after ($M = 0.1510$, SE = 0.0134), $t_{15} = 2.812$, $p < 0.013$ was also evident. Also, at alpha2 band, in the occipital regions, the significant decrease at O1 from before ($M = 0.1399$, SE = 0.0156) and after ($M = 0.1243$, SE = 0.0111), $t_{15} = 2.256$, $p < 0.039$; and at O2 from before ($M = 0.1383$, SE = 0.0137) and after ($M = 0.1203$, SE = 0.0104), $t_{15} = 3.283$, $p < 0.005$ was revealed. There was a large decrease in relative difference from before to after by 12% (P3), 18.4% (P4), 11.2% (O1) and 13% (O2) than at any other electrode and stimulation. The three-way ANOVA revealed a significant interaction between exposure/control and sessions for P3 electrode $F_{1,31} = 11.918$, $p < 0.002$ and the main factor before/after $F_{1,31} = 5.230$, $p < 0.029$. At P4 electrode, a significant difference between exposure/control and sessions was $F_{1,31} = 14.827$, $p < 0.001$ and before/after $F_{1,31} = 4.406$, $p < 0.044$; O1 revealed $F_{1,31} = 9.346$, $p < 0.005$ (exposure/control and sessions); and O2 $F_{1,31} = 13.071$, $p < 0.001$ (Table 1). The *t*-test results for 13-Hz stimulation in beta1 band revealed no significant differences at any electrode, as shown in Fig. 5. For the first MF exposure session, the *t*-test results revealed a significant increase at Fp1, Fp2, F7, F3 and C3 for 13-Hz stimulation in beta1 band. At F7 before, $t_{15} = -2.798$, $p < 0.014$; F3 before $t_{15} = -2.659$, $p < 0.018$; and C3 before $t_{15} = -2.391$, $p < 0.030$. There was an increase from before to after by 10.1% (Fp1), 8% (Fp2), 8.4% (F7), 10.8% (F3) and 9.3% (C3). The ANOVA results revealed significant differences between before and after main factors at Fp1 $F_{1,31} = 12.852$, $p < 0.001$; Fp2 $F_{1,31} = 7.058$, $p < 0.012$; F7 $F_{1,31} = 5.730$, $p < 0.0001$ (Table 1). In first MF exposure beta1 band (13 Hz), ANOVA's significant results for before and after main factor, were very similar to *t*-test's results (Fig. 5; Table 1).

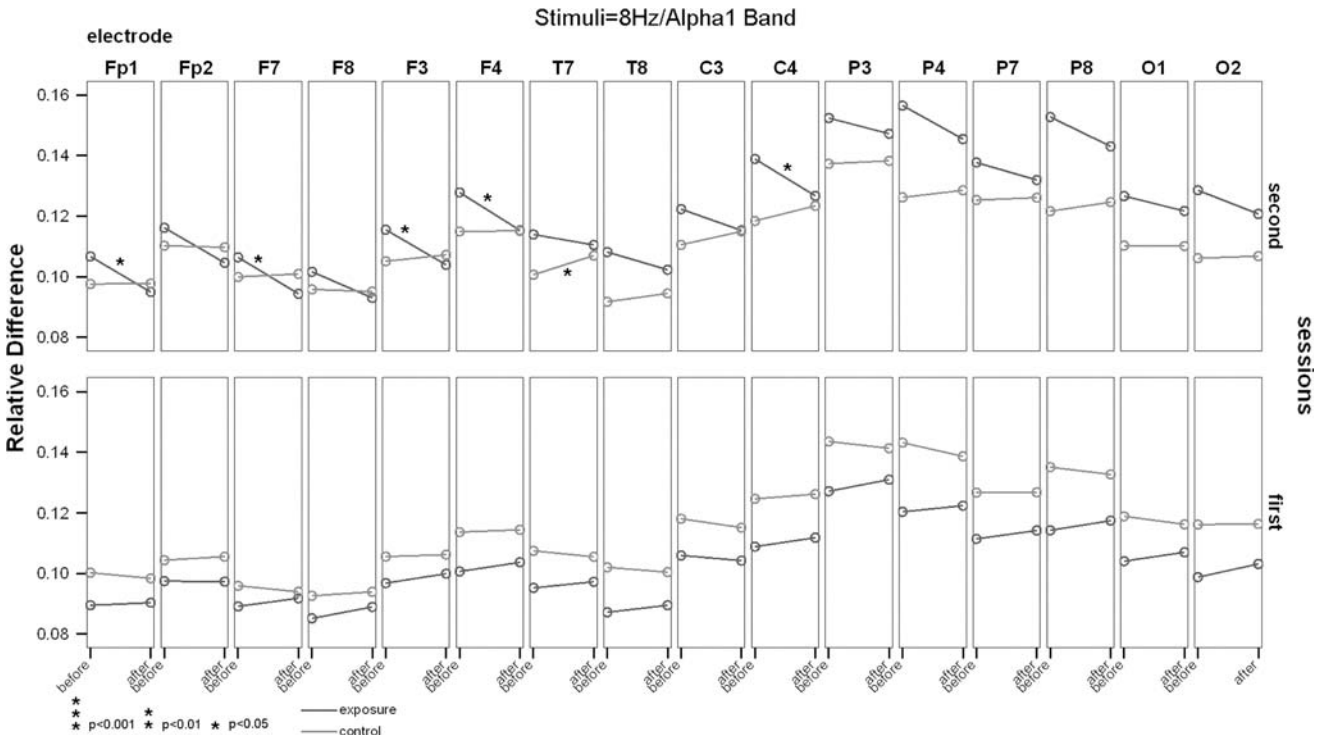

Fig. 3 The mean relative difference (*y*-axis) within alpha1 EEG band is represented as 'before' and 'after' (*x*-axis) MF condition (exposure/control) over 16 EEG electrodes (*columns*) at 8.33 Hz stimulus. The MF condition was represented by MF exposure (*darker colour line*) and MF control (*lighter colour line*) at first and second sessions (*rows*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$) decrease from before to after MF exposure, in relative alpha1 band power, was mainly exhibited over the frontal regions during the second session

3.2 MF control followed by MF exposure results

For the second MF exposure session, the *t*-tests results for 8.33 Hz stimulation in alpha1 band revealed a significant decrease from before to after at electrodes Fp1, F7, F3, F4 and C4: F7 $t_{16} = 2.120$, $p < 0.050$; F3 $t_{16} = 2.862$, $p < 0.011$; F4 $t_{16} = 2.682$, $p < 0.016$; and C4 $t_{16} = 2.872$, $p < 0.011$ (Fig. 3). There was a decrease in relative difference from before to after by 11.1% (Fp1), 11.3% (F7), 10% (F3), 9.8% (F4) and 8.8% (C4). The ANOVA results (Table 1) indicated a significant difference at: F7 $F_{1,31} = 6.485$, $p < 0.016$ (exposure/control and sessions) and $F_{1,31} = 4.485, p < 0.042$ (before/after and sessions); F3 $F_{1,31} = 4.524$, $p < 0.041$ (exposure/control and sessions) and $F_{1,31} = 4.297, p < 0.047$ (before/after and sessions); F4 $F_{1,31} = 11.554, p < 0.002$ (exposure/control and sessions); and C4 $F_{1,31} = 5.121$, $p < 0.031$ (exposure/control and sessions) and $F_{1,31} = 6.035$, $p < 0.020$ (before/after and sessions). For the second MF exposure session, the *t*-test results revealed a significant increase from before to after 10-Hz exposure in alpha2 band at F4, $t_{16} = -2.130$, $p < 0.049$, as shown in Fig. 4. ANOVA revealed a significant difference for the interaction between exposure/control and session's factor, $F_{1,31} = 11.043$, $p < 0.002$ (Table 1). For 13-Hz stimulation, there was no significant difference (Fig. 5).

3.3 Bonferroni alpha-correction for multiple ANOVA and post-hoc *t*-tests

Alpha-adjusted procedure is a frequently used method to control the inflated *type I error* due to repeated measures. The post-hoc analysis was performed using Bonferroni's corrected alpha rate, which needed to be conducted due to multiple *t* and ANOVA tests. Initially, for the multiple *t*-tests, there were 16 electrodes, five bands/stimulations, two conditions (exposure/control) and two sessions (first/second). As a result, the alpha rate of $p < 0.05$ was corrected to $p < 0.05/320 = 0.000156$. No significant differences were observed as a result of this correction.

On the other hand, the ANOVA tests conducted for 16 electrodes and five bands/stimulations, resulted in the corrected alpha rate of $p < 0.05/80 = 0.000625$. Clearly, the ANOVA's new alpha rate was higher than the *t*-test's by a factor of 4. Under this alpha rate correction (Table 1), the significant difference was revealed at: beta1 (13 Hz) band/simulation F7 electrode, $F_{1,31} = 15.73, p < 0.0005$ for main 'before/after' effect factor; alpha2 (10 Hz) T8 $F_{1,31} = 16.81$ $p < 0.0005$ and P7 $F_{1,31} = 17.25$ $p < 0.0005$ for 'condition × sessions' factor; alpha1 (8.33 Hz) P7 $F_{1,31} = 16.40$ $p < 0.0005$ and P8 $F_{1,31} = 15.00$ $p < 0.0005$ for 'condition × sessions' factor.

Table 1 The multiple repeated three-way ANOVA mixed design (within and between-subject) test results

Three-way ANOVA	Beta2 (16.66 Hz)		Beta1 (13 Hz)		Alpha2 (10 Hz)		Alpha1 (8.33 Hz)		Theta (4 Hz)	
$F_{1,31}$	F	p	F	p	F	p	F	p	F	p
Fp1	$4.585^{b\cdot c}$	$0.040^{b\cdot c}$	12.85^{a}	0.001^{a}	$11.12^{b\cdot c}$	$0.002^{b\cdot c}$	$5.667^{b\cdot c}$	$0.024^{b\cdot c}$		
Fp2	$5.287^{b\cdot c}$	$0.028^{b\cdot c}$	7.058^{a}	0.012^{a}	$10.18^{b\cdot c}$	$0.003^{b\cdot c}$	$8.400^{b\cdot c}$	$0.007^{b\cdot c}$		
	$5.529^{a\cdot c}$	$0.025^{a\cdot c}$								
F7			**15.73**^{a}	**0.0005**^{a}	$11.14^{b\cdot c}$	$0.002^{b\cdot c}$	$6.485^{b\cdot c}$	$0.016^{b\cdot c}$	$10.86^{b\cdot c}$	$0.002^{b\cdot c}$
							$4.485^{a\cdot c}$	$0.042^{a\cdot c}$	5.066^{a}	0.032^{a}
F8	4.781^{b}	0.036^{b}			$10.98^{b\cdot c}$	$0.002^{b\cdot c}$	$5.565^{b\cdot c}$	$0.025^{b\cdot c}$	$7.788^{b\cdot c}$	$0.009^{b\cdot c}$
F3			8.140^{a}	0.008^{a}	$8.727^{b\cdot c}$	$0.006^{b\cdot c}$	$4.524^{b\cdot c}$	$0.041^{b\cdot c}$	$10.99^{b\cdot c}$	$0.002^{b\cdot c}$
			$5.146^{b\cdot a}$	$0.030^{b\cdot a}$			$4.297^{a\cdot c}$	$0.047^{a\cdot c}$	5.944^{a}	0.021^{a}
F4	$8.728^{b\cdot c}$	$0.006^{b\cdot c}$	$7.960^{b\cdot a}$	$0.008^{b\cdot a}$	$11.04^{b\cdot c}$	$0.002^{b\cdot c}$	$11.55^{b\cdot c}$	$0.002^{b\cdot c}$	$8.751^{b\cdot c}$	$0.006^{b\cdot c}$
									5.448^{a}	0.026^{a}
T7			$4.449^{b\cdot a}$	$0.043^{b\cdot a}$	$1.564^{b\cdot c}$	$0.002^{b\cdot c}$	$5.992^{b\cdot c}$	$0.020^{b\cdot c}$	$9.390^{b\cdot c}$	$0.004^{b\cdot c}$
					4.473^{c}	0.043^{c}				
T8					**16.81**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$			$6.975^{b\cdot c}$	$0.013^{b\cdot c}$
									$4.157^{b\cdot a}$	$0.050^{b\cdot a}$
C3	4.300^{a}	0.047^{a}			$7.898^{b\cdot c}$	$0.008^{b\cdot c}$			$13.75^{b\cdot c}$	$0.001^{b\cdot c}$
					4.278^{c}	0.047^{c}				
C4			$4.176^{b\cdot a}$	$0.050^{b\cdot a}$	$13.64^{b\cdot c}$	$0.001^{b\cdot c}$	$5.121^{b\cdot c}$	$0.031^{b\cdot c}$	$8.825^{b\cdot c}$	$0.006^{b\cdot c}$
							$6.035^{a\cdot c}$	$0.020^{a\cdot c}$		
P3					$11.92^{b\cdot c}$	$0.002^{b\cdot c}$	$7.381^{b\cdot c}$	$0.011^{b\cdot c}$	$9.911^{b\cdot c}$	$0.004^{b\cdot c}$
					5.230^{a}	0.029^{a}			5.222^{a}	0.029^{a}
P4					$14.83^{b\cdot c}$	$0.001^{b\cdot c}$	$6.606^{b\cdot c}$	$0.015^{b\cdot c}$	$10.45^{b\cdot c}$	$0.003^{b\cdot c}$
					4.406^{a}	0.044^{a}				
P7	4.156^{c}	0.050^{c}			**17.25**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$	**16.40**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$	$10.93^{b\cdot c}$	$0.002^{b\cdot c}$
									$5.688^{b\cdot a}$	$0.023^{b\cdot a}$
P8					$13.41^{b\cdot c}$	$0.001^{b\cdot c}$	**15.00**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$	$8.632^{b\cdot c}$	$0.006^{b\cdot c}$
					4.229^{a}	0.048^{a}				
O1					$9.346^{b\cdot c}$	$0.005^{b\cdot c}$	$6.837^{b\cdot c}$	$0.014^{b\cdot c}$	$8.614^{b\cdot c}$	$0.006^{b\cdot c}$
O2	$5.918^{b\cdot c}$	$0.021^{b\cdot c}$			$13.07^{b\cdot c}$	$0.001^{b\cdot c}$	$9.671^{b\cdot c}$	$0.004^{b\cdot c}$	$8.492^{b\cdot c}$	$0.007^{b\cdot c}$
									$4.679^{b\cdot a}$	$0.038^{b\cdot a}$

The ANOVA main effect and interaction between factors were represented as: before/after^{a}; condition^{b}; sessions^{c}; before/after × sessions$^{a\cdot c}$; condition × before/after$^{b\cdot a}$; and condition × sessions$^{b\cdot c}$. For all the results, the degree of freedom was adjusted by Greenhouse and Geisser's epsilon when appropriate. The tests that showed no significant ($p > 0.05$) differences are denoted by empty spaces. The ANOVA values highlighted in bold show the Bonferroni's alpha-corrected significant differences ($p < 0.000625$)

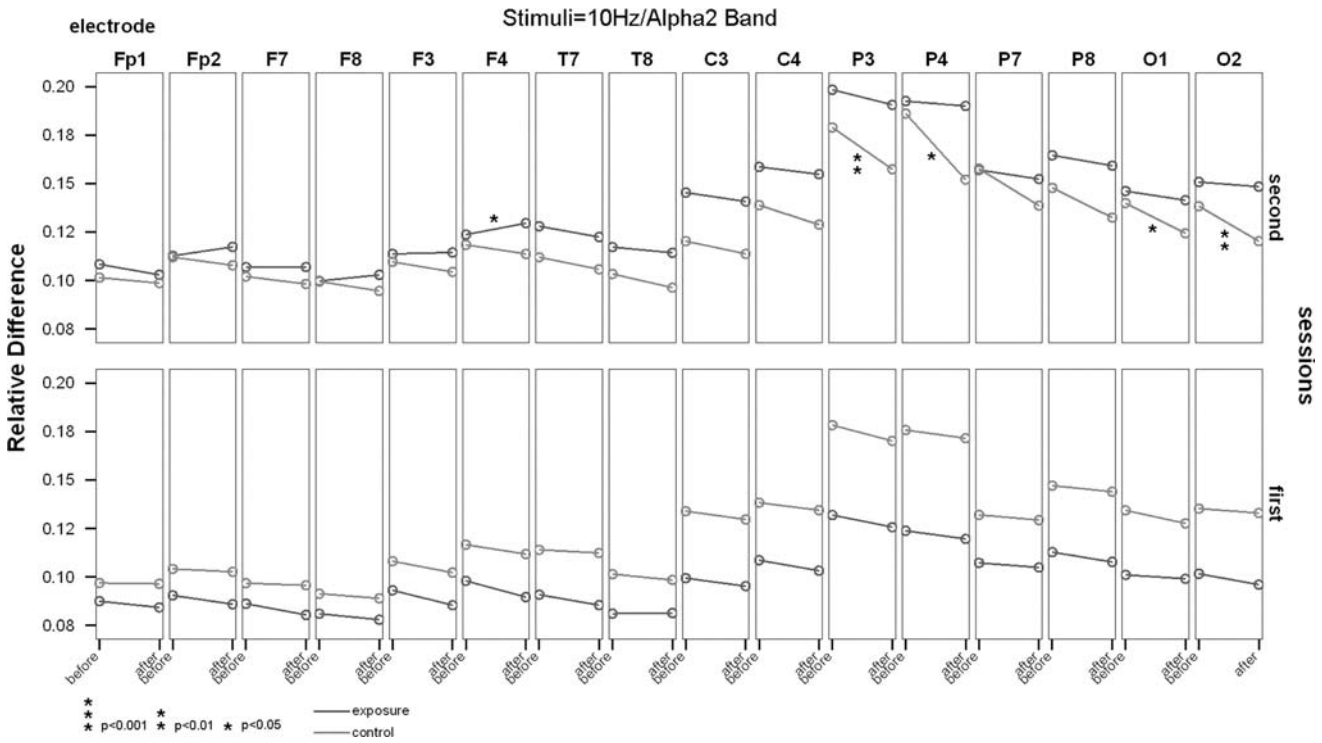

Fig. 4 The mean relative difference (*y*-axis) within alpha2 EEG band is represented as 'before' and 'after' (*x*-axis) MF condition (exposure/control) over 16 EEG electrodes (*columns*) at 10-Hz stimulus. The MF condition was represented by MF exposure (*darker colour line*) and MF control (*lighter colour line*) at first and second sessions (*rows*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$ and 0.01) decrease from before to after MF control, in relative alpha2 band power, was mainly exhibited over the parietal and occipital regions during the second session

4 Discussion

The statistical tests have been conducted to find any possible alteration of human EEG responses due to ELF MF exposures. The Bonferroni's corrected alpha rate on multiple ANOVA tests was able to indicate the significant differences of the main effect test factors and its combined effects or interaction between factors, such as MF exposure/control versus sessions (first/second). In response to alpha-corrected ANOVA results, it has been revealed that the relative power in alpha1 (7.5–9.5 Hz) and alpha2 (9–11 Hz) bands, associated with 8.33 and 10 Hz MF exposures, were significantly ($p < 0.0005$) lower than at MF control over the temporal and parietal regions within the 10–16 min of first session MF exposure. In addition, at the MF exposures, the relative powers in alpha1 and alpha2 bands were significantly higher than at MF control of the second session MF exposure (60–65 min from the commencement of testing).

It is unknown why the relative power in alpha (7.5–11 Hz) bands was 'suppressed' or decreased, associated with 8.33 and 10 Hz stimulus throughout the first 10–16 min MF exposure period. Its behaviour was consistent with our pilot study and other studies. The results from our previous pilot study revealed a 'marginal' significant decrease in MF exposure compared to MF control, found in the alpha1 EEG band (7.5–9.5 Hz) at the vertex head position, where MF stimulation was applied at the alpha1 frequency of 174 μT/8.33 Hz [8]. The consistent results with our study were also reported in another study with a decrease in the alpha (8–13 Hz) EEG activity at the occipital region after 2-s MF exposure of 0–60 Hz/20–100 μT [2]. Another study revealed a decrease in global field power and no indication in any frontal alpha asymmetry [ratio of right (F4) and left (F3) frontal powers] [20]. Our future analysis could perhaps employ similar method such as the EEG hemispheric asymmetry of anterior–posterior (A–P) regions and inter/intra hemispheric coherence. This type of analysis method was undertaken by other authors to investigate the photic stimulation responses on EEG activity [17]. The recent double-blind counter-balanced study (20 subjects), among the first to assess EEG activity changes during a weak ELF PEMF exposure, revealed that the alpha (8–13 Hz) EEG band was significantly lower over the occipital region after the first 5 min of MF, related to order of MF-sham versus sham-MF condition [6]. Our study has also shown that alpha1 and alpha2 bands were also significantly lower, but over the temporal and parietal regions (instead of occipital) during

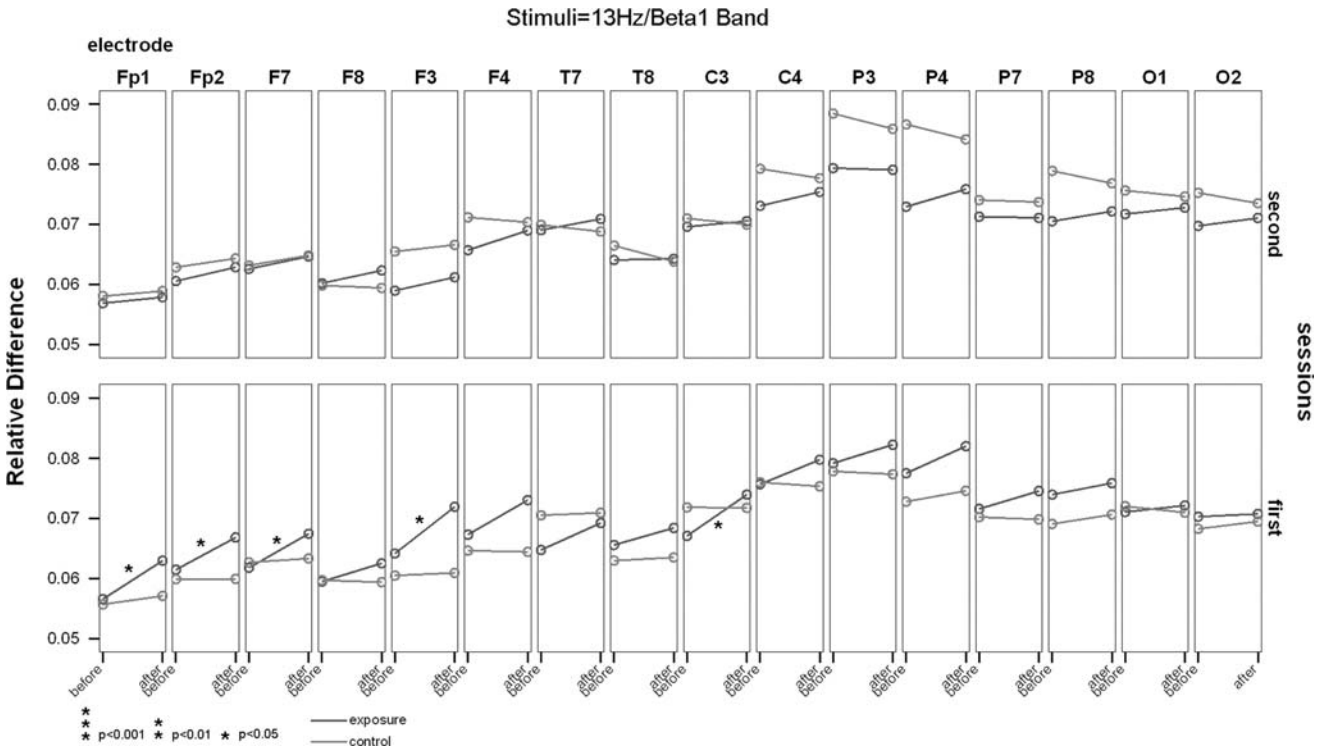

Fig. 5 The mean relative difference (*y*-axis) within beta1 EEG band is represented as 'before' and 'after' (*x*-axis) MF condition (exposure/control) over 16 EEG electrodes (*columns*) at 13 Hz stimulus. The MF condition was represented by MF exposure (*darker colour line*) and MF control (*lighter colour line*) at first and second sessions (*rows*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$) increase from before to after MF exposure, in relative beta1 band power, was mainly exhibited over the frontal regions during the first session

the first session (after 10–16 min). Some of the evident differences between ours and [6] Cook et al.'s protocol and analysis were: PEMF (0–500 Hz) versus ELF (4–16.66 Hz); Cook's alpha band (8–13 Hz) was much wider in frequency range compared to our narrow alpha1, alpha2 and beta1 bands, resulting in frequency range from 7.5 to 14 Hz; and ANOVA measures were analysed by four sets of EEG electrodes (O1, Oz, O2; P3, Pz, P4; CPz, C3, Cz, C4; FCz, F3, Fz, F4) versus 16 individual EEG electrodes in this study. The 15-min duration and the 'session' counter-balanced design (MF exposure/control) was very similar in both studies. For our future statistical analysis, we should adopt the 4–5 sets of EEG electrodes which could definitely increase the altered-alpha rate value, and therefore improve the significant difference of our results.

It is also unknown why the relative powers in alpha1 and alpha2 bands, associated with 8.33 and 10 Hz stimulus, were significantly higher than at MF control of the second session MF exposure. These results were contradictory with our pilot study [8], but consistent with many other previous studies [4, 5] on EEG responses, which revealed that the alpha activity was significantly higher over the occipital region, and marginally higher over the parietal electrodes at 15-min post exposure. The other consistencies in the alpha band increase at MF exposure compared to MF control were also reported in the following studies: a decrease in the delta (1–3 Hz) and theta (4–7 Hz) (frontal/central/parietal regions) and an increase in the alpha (7–13 Hz) and beta (14–25 Hz) in the respective occipital and frontal regions (45-Hz MF) [14]; an increase in the alpha (10 Hz) EEG activity at the central region after 10-min MF exposure of 10 Hz/40 μT and 1.5 Hz/20 μT [3]; an increases in the spectral power mainly at higher than 10 Hz EEG frequencies at the central, parietal and occipital regions due to 2-s light and EMF exposures to 80 μT/1.5 Hz and 10 Hz [15]; and a significant increase in the alpha (8–13 Hz) EEG activity over occipital region due to a 90 min MF exposure 50 Hz/80 μT [11].

Considering that our ANOVA tests findings were not able to determine whether there was a significant increase or decrease (Fig. 6) from before to after MF exposure or control at the specific session, the post-hoc analysis was conducted using multiple paired samples two-tailed *t*-tests and later employing the Bonferroni's corrected alpha rate. From the combination of the ANOVA and *t*-test results, it was found that the beta1 (12–14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at left frontal region (Figs. 5, 6; Table 1). This particular finding was also consistent with previous

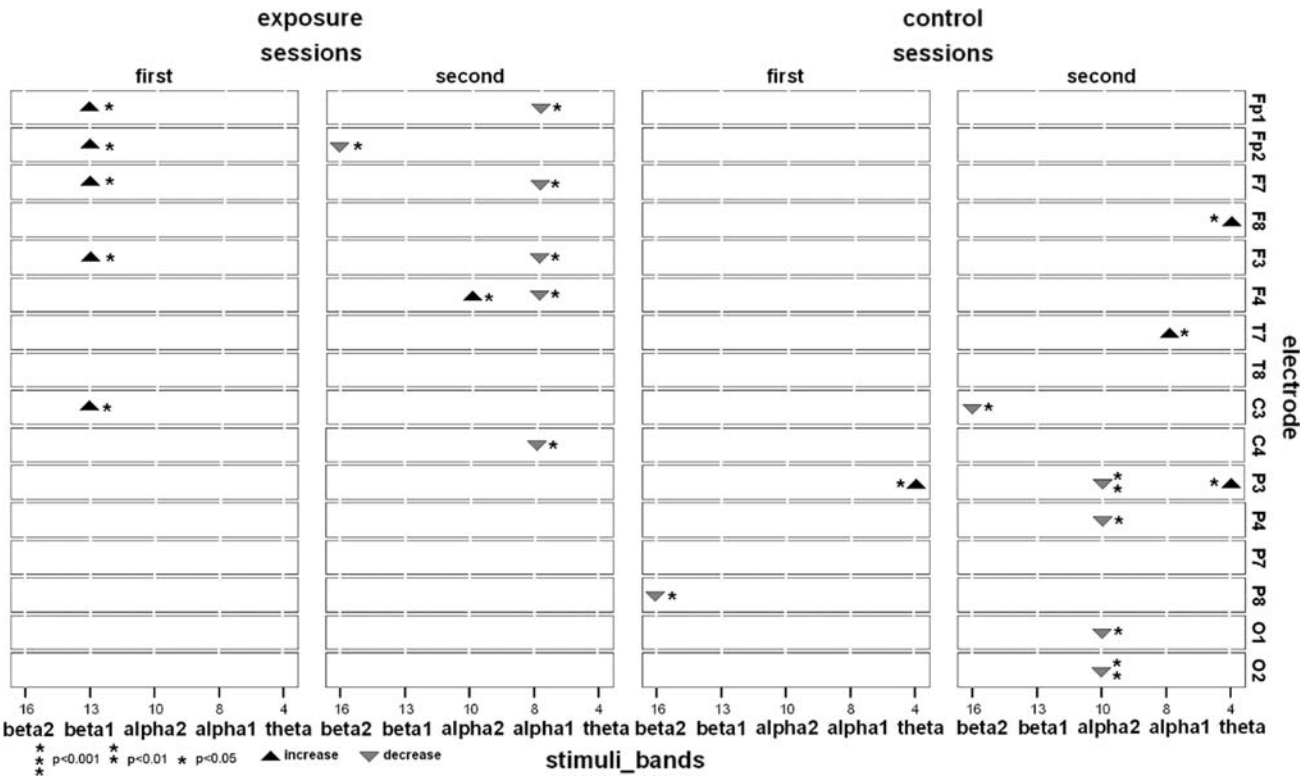

Fig. 6 A graphical representation of the relative increase (*darker colour pointer facing up*) and decrease (*lighter colour pointer facing down*) at each MF stimulus and EEG band (*x*-axis) over the 16 EEG electrodes (*y*-axis). The MF condition was represented by MF exposure (first two columns on the left-hand side) and MF control (last two columns on the right-hand side) at first and second sessions (*columns*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$) increase from before to after MF exposure during the first session was exhibited in relative beta1 band power over the frontal regions. On the other hand, the significant decrease from before to after MF exposure (second session) was revealed over frontal region at alpha1 band and MF control (second session) over the parietal and occipital regions at alpha2 band

studies, in particular [14] Lyskov et al. study where an increase in beta band (14–25 Hz) was evident in the frontal region during the 45-Hz MF exposure [13] and Hausser et al. study which revealed a significant increases in the beta (12.5–25 Hz) EEG bands over the occipital region after the 20-min, 3-Hz MF exposure.

The Bonferroni's corrected alpha rate *t*-tests did not reveal any significant differences due to an extremely low alpha rate of $p < 0.000156$. However, it did reveal a significant ($p < 0.05$) increase from before to after MF exposure during the first session in relative beta1 band power over the frontal regions (Figs. 5, 6). The significant decrease from before to after MF exposure (second session) was also revealed over the frontal region at alpha1 band and from before to after MF control (second session) over the parietal and occipital regions at alpha2 band (Fig. 6).

Our beta1 and alpha2 band findings indicate that humans could exhibit significant changes in the EEG during the MF exposure, such as by having the natural ability to detect ELF MFs [16]. The beta2 frequency (16.66 Hz) used in this study was also used as the frequency within the beta1 band of the EEG in [19] Sastre et al. study which claimed that beta1 band exhibits a close temporal association with REM sleep and has a reciprocal relationship with delta activity during NREM sleep. Griefahm et al. study also utilised 16.7-Hz MF exposure [12]. However, this study on the effects of 16.66 Hz on beta2 EEG band failed to present any evident and significant changes.

In vitro experimental study has observed in 'real-time' the intracellular synchronised bioelectric activity in neurons, from the brain ganglia of the snail *Helix aspersa*, under (1–50 Hz/15 mT) [1]. This study demonstrated the ability of low frequency sinusoidal weak MF to promote synchronisation in the bioelectric activity in neurons. The results revealed that the decrease of neuron firing frequency and synchronisation can be observed with an increasing static and ELF MF. In vivo studies, similar EEG synchronisation has been demonstrated using the non-invasive direct stimulation of the brain via pulsed MF, known as transcranial magnetic stimulation (TMS) [17]. However, there has not been enough research on EEG

synchronisation from sinusoidal 'weaker' MF stimulation which has been demonstrated in this study.

Over the years, the research examining the effects of MF on human performance and physiology has produced inconsistent results [22]. Our 'lack' of significant findings due to Bonferroni's adjustment could be based on Podd et al. study [18] which reported negative findings that 0.2-Hz MF affected simple reaction time in humans, and whereas a 0.1-Hz field did not. They discussed the various issues which could improve the significance of their results. One of those issues raised was the degree of statistical power. In order to increase the statistical power is to increase the number of subjects which in our study was minimal. Also, adjusting the probability of a type I error (alpha level) or by reducing the number of relevant experimental design factors could increase the statistical power. Thus, any other future research studies including ours must give serious thought to minimise the error of variance and maximise statistical power.

Thus, an increased sample size, adopted EEG hemispheric asymmetry method and alternative alpha-adjustment tests could improve on the significant differences of these findings in the future studies.

5 Conclusion

The results from this study have shown a two-folding outcome which is equally consistent and inconsistent with our pilot and other studies, conducted over the last several years. In order to investigate whether weak MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within the corresponding EEG bands, this study's consistency with our pilot study has revealed that the relative power in alpha bands, associated with 8.33 and 10 Hz MF exposures was significantly lower than at MF control over the temporal and parietal regions within the first session of MF exposure. However, at the second session of MF exposure, the relative power in alpha bands was significantly higher than at MF control. Also, it was found that the beta1 (12–14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at frontal region.

The final outcome of our result has shown that it is possible to alter the human EEG activity of alpha and beta bands when exposed to MF at associated EEG frequency bands, depending on the order and period of MF conditions. This type of EEG synchronisation of driving alpha and beta EEG by alpha and beta sinusoidal MF stimulation, demonstrated in this study, could possibly be applied as therapeutic treatment(s) of particular neurophysiological abnormalities such as sleep and psychiatric disorders. In future, authors expect that the EEG synchronisation can effectively be demonstrated by either flickering of lights [10] and/or MF at ELF.

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References

1. Azanza MJ, Calvo AC, Moral AD (2002) Evidence of synchronisation of neural activity of molluscan brain ganglia induced by alternating 50 Hz applied magnetic field. Electromagn Biol Med 21(3):209–220
2. Bell GB, Marino AA, Chesson A, Struve FA (1991) Human sensitivity to weak magnetic fields. Lancet 338:1521–1552
3. Bell GB, Marino AA, Chesson A (1994) Frequency-specific responses in the human brain caused by electromagnetic fields. J Neurol Sci 123:26–32
4. Cook CM, Thomas AW, Prato FS (2002) Human electrophysiological and cognitive effects of exposure to ELF magnetic and ELF modulated RF and microwave fields: a review of recent studies. Bioelectromagnetics 23:144–157
5. Cook CM, Thomas AW, Prato FS (2004) Resting EEG is affected by exposure to a pulsed ELF magnetic field. Bioelectromagnetics 25:196–203
6. Cook CM, Thomas AW, Keenliside L, Prato FS (2005) Resting EEG effects during exposure to a pulsed ELF magnetic field. Bioelectromagnetics 26:367–376
7. Cvetkovic D (2005) Electromagnetic and audio-visual stimulation of the human brain at extremely low frequencies. PhD Thesis, RMIT University, Melbourne, Australia
8. Cvetkovic D, Cosic I (2006) Automated ELF magnetic field stimulation of the human EEG activity. Integr Comput Aided Eng 13(4):313–328
9. Cvetkovic D, Cosic I (2007) Modelling and design of extremely low frequency uniform magnetic field exposure apparatus for in vivo bioelectromagnetic studies. In: Proceedings of the 29th annual international conference IEEE engineering in medicine and biology society (EMBS), Lyon, France, pp 1675–1678
10. Cvetkovic D, Cosic I (2009) EEG inter/intra-hemispheric coherence and asymmetric responses to visual stimulations. Med Biol Eng Comput. doi:10.1007/s11517-009-0499-z
11. Ghione S, Seppia CD, Mezzasalma L, Bonfiglio L (2006) Effects of 50 Hz electromagnetic fields on electroencephalographic alpha activity, dental pain threshold and cardiovascular parameters in humans. Neurosci Lett 382:112–117
12. Griefahn B, Kunemund C, Blaszkewicz M, Golka K, Degen G (2002) Experiments on effects of an intermittent 16.7-Hz magnetic field on salivary melatonin concentrations, rectal temperature and heart rate in humans. Int Arch Occup Environ Health 75:171–178
13. Hausser K, Tellschaft D, Thoss F (1997) Influence of an alternating 3 Hz magnetic field with an induction of 0.1 mT on chosen parameters of the human occipital EEG. Neurosci Lett 239:57–60
14. Lyskov EB, Juutilainen J, Jousmaki V, Partanen J, Medvedev S, Hanninen O (1993) Effects of 45-Hz magnetic fields on the functional state of the human brain. Bioelectromagnetics 14:87–95
15. Marino AA, Bell GB, Chesson A (1996) Low-level EMFs are transduced like other stimuli. J Neurol Sci 144:99–106
16. Marino AA, Nilsen E, Chesson AL Jr, Frilot C (2004) Effect of low-frequency magnetic fields on brain electrical activity in human subjects. Clin Neurophysiol 115:1195–1201

17. Paus T, Sipila PK, Strafella AP (2001) Synchronisation of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. J Neurophysiol 86(4):1983–1990
18. Podd JV, Whittington CJ, Barnes GRG, Page WH, Raply BI (1995) Do ELF magnetic fields affect human reaction time. Bioelectromagnetics 16:317–323
19. Sastre A, Graham C, Cook MR (2000) Brain frequency magnetic fields alter cardiac autonomic control mechanisms. Clin Neurophysiol 111:1942–1948
20. Stevens P (2006) Affective response to 5 μT ELF magnetic field-induced physiological changes. Bioelectromagnetics 28(2):109–114
21. Thomas AW, Kavaliers M, Prato FS, Ossenkopp KP (1997) Antinociceptive effects of pulsed magnetic fields in the land snail: *Cepaea nemoralis*. Neurosci Lett 222:107–110
22. Whittington CJ, Podd JV (1996) Human performance and physiology: a statistical power analysis of ELF electromagnetic field research. Bioelectromagnetics 17:274–278